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TITLE: Low-Intensity Vibration as a Treatment for Traumatic Muscle Injury

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CONTRACTING ORGANIZATION: University of Illinois
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14. ABSTRACT (~200 words of most sig finding during this period)
Traumatic musculoskeletal injuries are among the most common injuries experienced during military combat. Poor healing of traumatic muscle injuries is associated with impaired muscle function, joint stiffness and loss of mobility. Our long-term goal is to develop a device and treatment protocol that provide a safe, inexpensive, and easy to apply treatment that will help to restore normal muscle and joint function to injured military personnel. In this report, we provide preliminary data indicating a trend towards improved healing with LIV. We observed a trend towards a larger fiber area and increased angiogenesis in muscles from LIV-treated mice vs. controls. We have initiated additional experiments to follow up on these findings. Furthermore, initial in vitro studies in macrophages (Mp) demonstrated that these cells are responsive to the LIV signals and that LIV downregulates the expression of pro-inflammatory markers and upregulates the expression of pro-healing markers in Mp. Findings from continued work on this project will provide insight into the potential for LIV as a non-invasive and simple treatment for improving muscle healing, thereby reducing joint stiffness and increasing mobility of polytrauma patients.

15. SUBJECT TERMS
Skeletal muscle repair, low-intensity vibration, monocytes/macrophages, endothelial precursor cells, angiogenesis, myogenesis

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1. INTRODUCTION

Traumatic skeletal muscle injuries typically result in impaired muscle function, joint stiffness and loss of mobility, which, in turn, results in significant costs for rehabilitation, loss of time for work and reduced combat readiness. Unfortunately, effective treatments for improving the recovery of muscle function and joint mobility are lacking. The proposed Idea Development study is an early-stage investigation into a novel treatment of traumatic muscle injuries – mechanical stimulation via low-intensity vibration (LIV). Mechanical stimulation has an anabolic effect on musculoskeletal tissues, and mechanical stimulation via LIV has been shown to accelerate bone regeneration. Our preliminary data indicate that LIV reduces fibrosis and enhances muscle fiber growth following traumatic muscle injury in mice. Our data also indicate that LIV increases numbers of monocytes and macrophages (Mo/Mp) and endothelial precursor cells (EPC) in the blood; these cells are known to promote healing of muscle injuries. Thus, the central hypothesis of this study is that LIV improves healing of traumatic muscle injuries by increasing the activity of Mo/Mp and EPC in damaged muscle. We will address this hypothesis in three Specific Aims: First, we will determine the effectiveness of locally applied versus whole-body LIV for improving angiogenesis and muscle regeneration and reducing fibrosis. Second, we will determine the role of bone marrow-derived cells (BMDC) in LIV-induced improvements in muscle healing. Third, we will identify specific cells that detect and transduce the LIV signal. If successful, LIV would provide an innovative, non-invasive and simple treatment for improving muscle healing and thereby reducing joint stiffness and increasing mobility of polytrauma patients.

2. KEYWORDS

Skeletal muscle repair, low-intensity vibration, monocytes/macrophages, endothelial precursor cells, angiogenesis, myogenesis

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goals of this project were divided into three Specific Aims:

1. Determine the effectiveness of locally applied versus whole-body low-intensity vibration (LIV) for improving muscle regeneration following traumatic injury.
2. Determine the role of bone marrow-derived cells (BMDC) in LIV-induced improvements in muscle healing.
3. Identify specific cells that detect and transduce the LIV signal.

Timeline and Cost

Activities CY	14	15	16
Local versus whole body LIV			
Role of BMDC in LIV healing			
Identify cells responsive to LIV			
Estimated Total Cost ($K)	**$258**	**$223**	**$227**

Figure 1. Timeline for completion of Specific Aims and associated estimated costs for each calendar year. The green bars denote the timeline for each of the three aims and the percentage of the aim completed is indicated in purple.

What was accomplished under these goals?

Specific Aim 1

In the Statement of Work for this project, the goal of Specific Aim 1 is to determine the effectiveness of locally applied versus whole-body low-intensity vibration (LIV) for improving muscle regeneration following traumatic injury. During this first year, much progress has been made on many Subtasks related to Major Task 1. Approval for animal experiments from the Department of Defense IACUC and the IACUC for the University of Illinois at Chicago (UIC) and Stony Brook University (SBU) was obtained, and the subcontract with SBU was negotiated and signed. In addition, a search for a post-doctoral fellow to perform the experiments associated with Specific Aims 1 and 2 resulted in hiring of Dr. Eileen Weinheimer-Haus in November. After Dr. Eileen Weinheimer-Haus was trained on the muscle injury technique, she promptly initiated preliminary experiments to optimize the whole body LIV signal. For these experiments, mouse gastrocnemius muscles were subjected to laceration injury and then mice were either subjected to daily bouts of whole-body LIV at one of two signals (0.4 g at 45 Hz or 0.2 g at 90 Hz) or handled identically without LIV treatment for controls. Fourteen days after injury, muscles were harvested and healing was assessed. In our preliminary results, no statistically significant differences in healing outcomes were found between LIV-treated mice and non-LIV control mice for both LIV protocols (Figure 2). While we did not see a statistically significant difference between treatment groups, there were trends towards a larger fiber area with both LIV protocols and increased angiogenesis in mice receiving the 0.2 g at 90 Hz LIV compared to controls. Interestingly, we have previously reported an increase in angiogenesis and improved healing with LIV in skin wounds of diabetic mice, who experience impaired angiogenesis and wound healing [1]. Thus, we initiated additional replicate experiments to follow up on these preliminary trends. For these experiments, mice were injured and subjected to either LIV or a non-LIV control. Gastrocnemius muscles from these mice have been collected and analysis of healing is in progress.

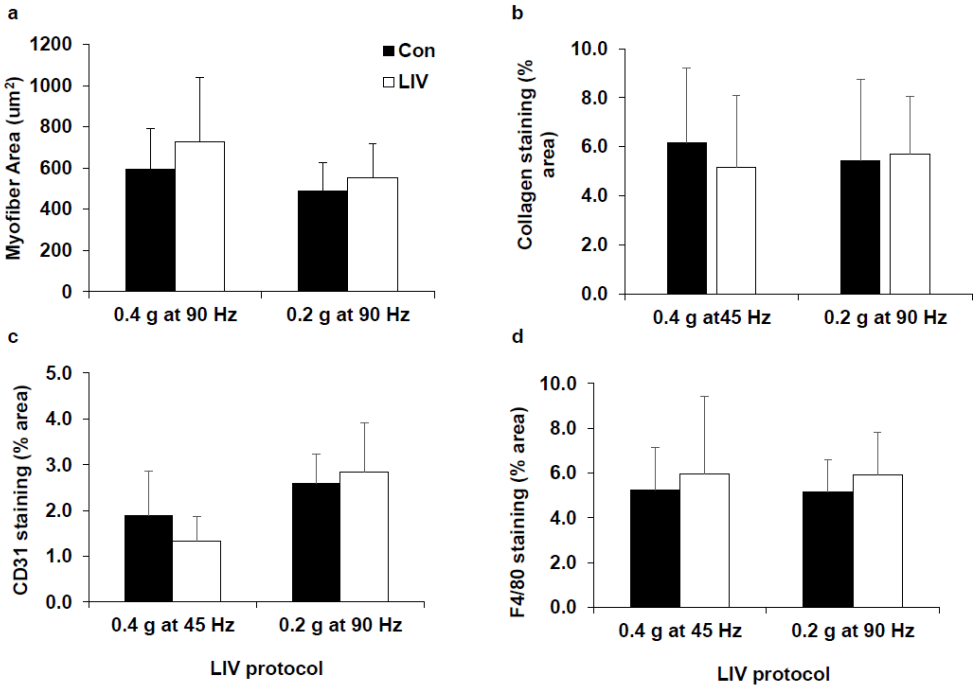

Figure 2. Effect of two different low-intensity vibration (LIV) signals on muscle healing following laceration injury. Mouse gastrocnemius muscles subjected to laceration injury and then mice either subjected to 30 minute bouts of whole-body LIV at one of two signals (0.4 g at 45 Hz or 0.2 g at 90 Hz) 5 d/wk or handled identically without LIV treatment for controls. Fourteen days after injury, muscles were harvested and healing was assessed in cryosections stained with (a) hematoxylin and eosin, (b) Masson's Trichrome, (c) CD31 (angiogenesis) and (d) F4/80 (macrophages). Data represent mean ± SD, n=7-19 per group.

The manufacturing of the device for local delivery of LIV, as outlined in Subtask 2, has been delayed due to parts (linear actuator) being on backorder (not received until March) and delays with the machine shop at SBU in manufacturing the support platform. Currently, the linear actuator and platform are assembled and the attachment fittings for the mice are being constructed. Dr. Judex is working diligently with the machine shop at Stoney Brook to complete assembly of the device. Thus, experiments for Specific Aim 1, Major Task 1, Subtasks 3 and 4 that apply LIV signals locally have been delayed. Additionally, Drs. Koh and Weinheimer-Haus have established a collaboration with Dr. Onur Bilgen at Old Dominion University to develop an alternative method for applying LIV locally. Dr. Bilgen is a mechanical engineer with an expertise in vibration energy. An initial prototype has been developed that uses a piezo electric buzzer to deliver LIV. Pending further testing, this collaboration may lead to the successful development of an alternative mode to deliver LIV locally to injured tissue.

Progress has also been made on Subtasks related to Major Task 2. Preliminary experiments were carried out to determine the effectiveness of early vs. late application of LIV for improving healing. For these experiments, mouse gastrocnemius muscles were subjected to laceration injury and mice then received either whole-body LIV (0.4 g at 45 Hz for 30 min/d, 5 d/wk) starting on day 14 post-injury or were handled identically without LIV treatment for controls. Muscles were collected at day 28 post-injury (14 days after initiation of LIV treatment) and healing was assessed. Preliminary results suggest that initiating LIV treatment 14 days post-injury does not appear to alter the healing response compared to control mice (Figure 3). The average minor fiber diameter (a measure of fiber size), muscle fibrosis and angiogenesis were not different between control and LIV groups.

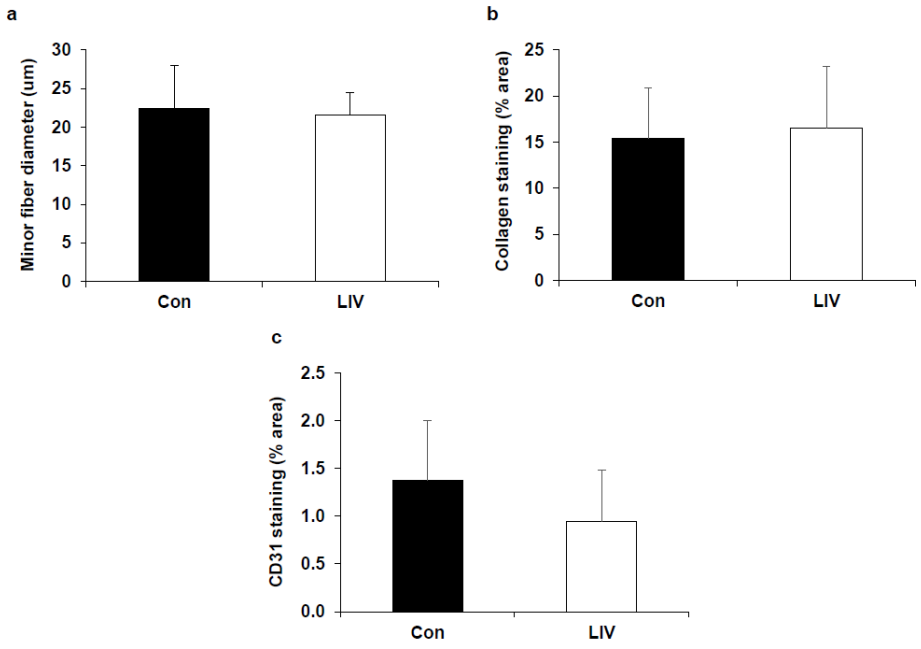

Figure 3. Effect of late application low-intensity vibration (LIV) signals on muscle healing following laceration injury. Mouse gastrocnemius muscles subjected to laceration injury and then mice subjected to either whole-body LIV (0.4 g at 45 Hz for 30 min/d, 5 d/wk) starting on day 14 post-injury or handled identically without LIV treatment for controls. On day 28 after injury, muscles were harvested and healing was assessed in cryosections stained with (a) hematoxylin and eosin, (b) Masson's Trichrome and (c) CD31 (angiogenesis). Data represent mean ± SD, n=6-8 per group.

Specific Aim 2

In the Statement of Work for this project, the goal of Specific Aim 2 is to determine the role of bone marrow-derived cells (BMDC) in LIV-induced improvements in muscle healing. Once the optimal LIV protocol is identified, we will initiate experiments for Specific Aim 2, Major Task 1, Subtask 1.

Specific Aim 3

In the Statement of Work for this project, the goal of Specific Aim 3 is to identify specific cells that detect and transduce the LIV signal. During the past year, in vitro LIV experiments were performed in murine macrophages (J774.1) to evaluate the effect of varying LIV signals on cell viability, proliferation, and phenotype. For these experiments, two different LIV signal frequencies (30 Hz or 100 Hz) were combined with two acceleration magnitudes (0.15 g or 1 g) to generate four distinct LIV signals. Murine macrophages were exposed to each of the four different LIV signals for 20 min per session for 2 sessions/day. Cell viability was unaffected by LIV, but proliferation was increased by each of the four LIV signals on days 1, 2, and 3 (Figure 4). The 100 Hz/0.15g signal appeared to be the most effective signal. In addition, mRNA expression of the pro-angiogenic growth factor VEGF and pro-healing growth factor TGF-β were higher in LIV-treated cells vs. non-LIV controls (Figure 5). For VEGF, the greatest increase in expression was with the 100 Hz/0.15g protocol, while there were no significant differences between individual LIV groups for TGF-β expression. Flow cytometric analysis of pro-inflammatory and pro-healing markers revealed a lower expression of the inflammatory markers IL-6, TNF-α, IFN-γ and GM-CSF (Figures 6) and a higher expression of the pro-healing makers IL-10 and M-CSF (Figure 7) in macrophages treated with LIV vs. control.

Collectively, these data demonstrate that macrophages are responsive to high-frequency oscillations applied at low intensities and that LIV downregulates the expression of pro-inflammatory markers and upregulates the expression of pro-healing markers in macrophages in vitro.

Data from these preliminary experiments were included in an abstract submitted to the American Society for Bone and Mineral Research annual meeting. Furthermore, Dr. Judex has sent an initial draft of the manuscript to Drs. Koh and Weinheimer-Haus and they are currently working on revisions. We plan to submit this paper to the Journal of Biomechanics.

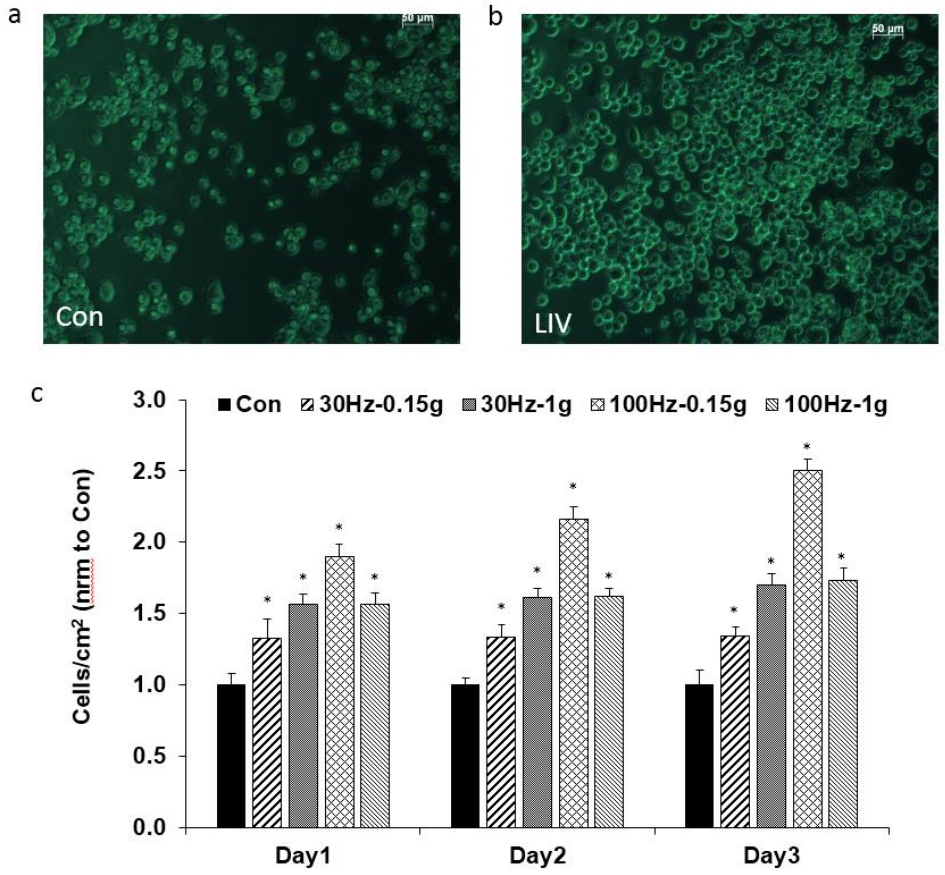

Figure 4: Effect of LIV on macrophage viability and proliferation in vitro. (a and b) Fluorescent images of J774.1 murine macrophages double stained with Calcein AM (green) and Ethidium Homodimer (red). Cells received non-LIV sham treatment (a) or LIV at 100Hz-0.15g (b). Viable cells in green demonstrate that LIV is not cytotoxic. (c) Cell density of macrophages under LIV and non-LIV conditions over 3 days. *p<0.05 vs Con.

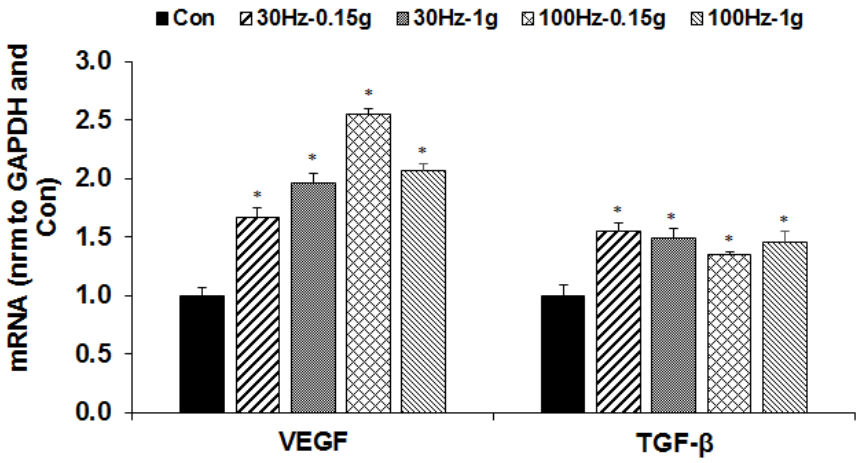

Figure 5: Effect of LIV on gene expression of growth factors in macrophages in vitro. Gene expression of pro-angiogenic growth factor VEGF and pro-healing growth factor TGF-β in macrophages treated with non-LIV sham (Con) or LIV. *p<0.05 vs Con.

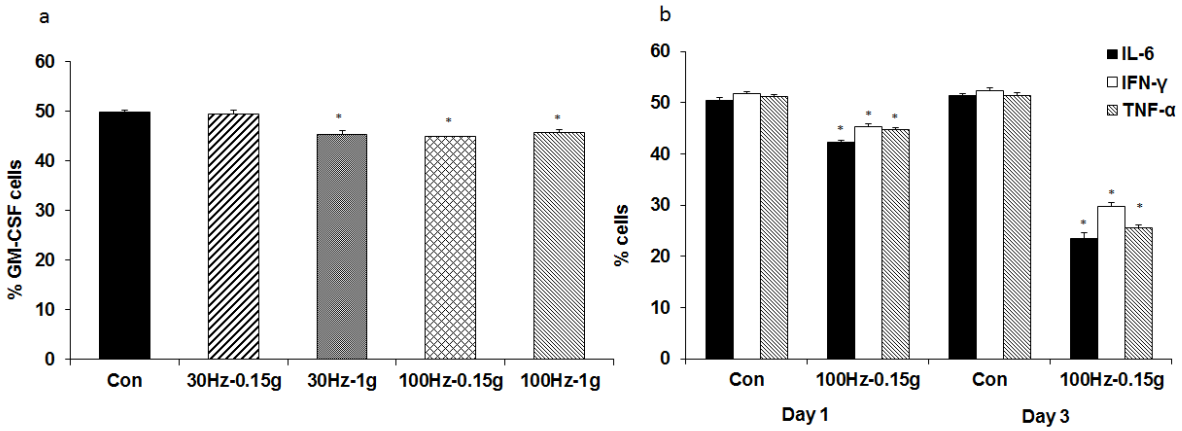

Figure 6: Effect of LIV on expression of pro-inflammatory markers in macrophages in vitro. Flow cytometric analysis of macrophages positive for pro-inflammatory markers (a) GM-CSF and (b) IFN-γ, IL-6, and TNF-α in non-LIV and LIV (100Hz-0.15g)-treated cells. *p<0.05 vs Con.

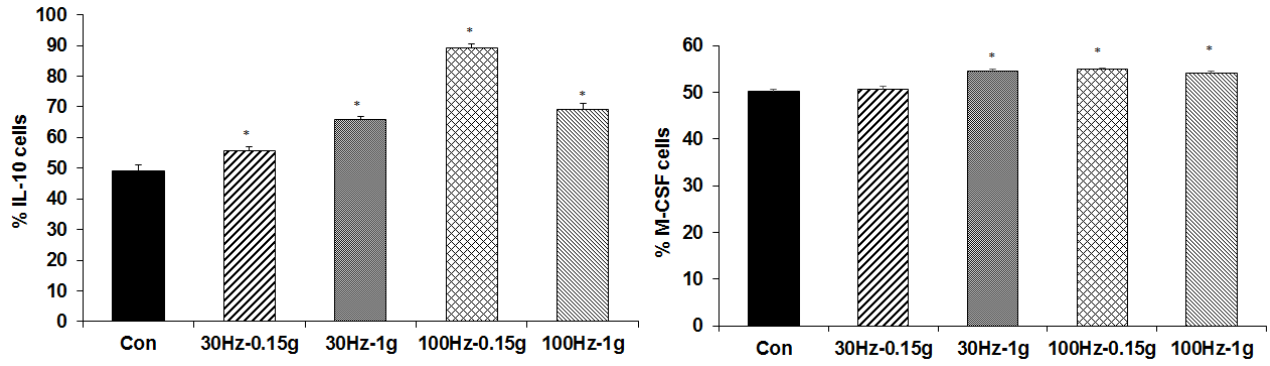

Figure 7: Effect of LIV on expression of pro-healing markers in macrophages in vitro. Flow cytometric analysis of macrophages positive for pro-healing markers (a) IL-10 and (b) M-CSF in non-LIV and LIV (100Hz-0.15g)-treated cells. *p<0.05 vs Con.

References

1. Weinheimer-Haus EM, Judex S, Ennis WJ, Koh TJ (2014) Low-intensity vibration improves angiogenesis and wound healing in diabetic mice. PLoS One 9: e91355.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals and objectives?

During the next reporting period, we plan the following:

1. Dr. Judex will recruit a graduate student to help carry out experiments in Specific Aim 3 and accomplish the tasks for year 1.
2. We will continue to work with our collaborators at Stony Brook University to finish the device for local delivery of LIV to the injured muscle.
3. We will complete analysis for the second cohort of experiments using the 0.2 g/90 Hz LIV signal.
4. We will initiate experiments for Specific Aim 1, Major Task 1, Subtasks 3 and 4, performing laceration injuries and applying optimized LIV signals locally to injured site or systemically via whole body vibration and assessing muscle regeneration and fibrosis in the injured muscles.
5. We will also initiate experiments for Specific Aim 2, Major Task 1, Subtask 1, determining whether LIV increases mobilization and homing of bone marrow derived cells (BMDC).
6. We will continue experiments for Specific Aim 3, Major Task 1, Subtask 1, determining whether LIV directly induces expression of pro-healing genes in BMDC, as well as Specific Aim 3, Major Task 2, Subtask 1, determining whether LIV directly induces secretion of growth factors associated with angiogenesis, regeneration, and fibrosis. C2C12 cells have been shipped to SBU (Subtask 4).

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Traumatic musculoskeletal injuries are among the most common injuries experienced during military combat and recovery from these injuries is typically prolonged and incomplete, leading to impaired muscle function, joint stiffness and loss of mobility. Unfortunately, effective treatments for improving the recovery of muscle structure and function and consequent joint mobility are lacking. Our long-term goal is to develop a device and treatment protocol that provide a safe, inexpensive, and easy to apply treatment that will help to restore normal muscle and joint function to injured military personnel. The proposed animal and cell culture studies will help to identify the optimal

methods for delivery of LIV signals to the damaged muscle and will begin to elucidate the mechanisms by which LIV signals improve healing. If successful, LIV would provide an innovative, non-invasive and simple treatment for improving muscle healing and thereby reducing joint stiffness and increasing mobility of polytrauma patients.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

1. Administrative negotiations between University of Illinois at Chicago and Stony Brook University for the subcontract took longer than expected and so the subcontract was in force until the end of this quarter. Therefore, the experiments in Specific Aim 3, Major Task 1, Subtask 1 were delayed. We have been working diligently to make up for lost time and get this part of the project back on schedule.

2. Parts needed to develop the device for local delivery of LIV were on backorder and did not arrive until March. Dr. Judex is currently working diligently with the machine shop at Stoney Brook to assemble the device. Thus, experiments for Specific Aim 1, Major Task 1, Subtasks 3 and 4 that apply LIV signals locally have been delayed. Additionally, Drs. Koh and Weinheimer-Haus have established a collaboration with Dr. Onur Bilgen at Old Dominion University to develop an alternative method for applying LIV locally.

3. Dr. Judex recruited a graduate student to follow up on the initial in vitro findings. However, the student did not perform up to standards and was let go. As a result, Dr. Judex increased his effort over the summer to perform the work as outlined in the grant and facilitate completion of the aims of the proposal for Year 1. He is actively searching for a replacement graduate student.

Changes that had a significant impact on expenditures

1. The amount expended in quarters 1 and 2 were below projections because of administrative delays in the negotiations of the sub-contract and hiring of the post-doctoral fellow in the first quarter, hence delaying the start of experiments.
2. The amount expended in quarter 3 was below projections because Dr. Judex had not yet hired a new graduate student and, thus, increase his effort to 1.56 summer months to facilitate completion of the aims of the proposal for Year 1.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations

Abstract was submitted to the American Society for Bone and Mineral Research annual meeting on data indicating macrophages are responsive to LIV, which downregulates expression of pro-inflammatory markers and upregulates expression of pro-healing (Specific Aim 3, Major Task 1, Subtask 1 and Major Task 2, Subtask 1). The manuscript is currently in preparation and an initial draft has been sent to Drs. Koh and Weinheimer-Haus who are working on revisions. We plan to submit this paper to the Journal of Biomechanics.

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Timothy Koh
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0001-6549-7060
Nearest person month worked:	1 academic month, 1 summer month
Contribution to Project:	Oversaw all aspects of the study including the preliminary in vivo LIV experiments and other activities at UIC

Name:	Stefan Judex
Project Role:	Co-I (SBU)
Researcher Identifier (e.g. ORCID ID):	0000-0002-4511-1535
Nearest person month worked:	2 summer months
Contribution to Project:	Oversaw in vitro experiments at SBU and is working with machine shop at SBU to manufacture device for local application of LIV

Name:	Eileen Weinheimer-Haus
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0001-5408-0024
Nearest person month worked:	12 calendar months
Contribution to Project:	Performed preliminary laceration and LIV experiments as well as analyses for Specific Aim 1.

Name:	Suphannee Pongkitwitoon
Project Role:	Graduate Student (SBU)
Researcher Identifier (e.g. ORCID ID):	0000-0003-0557-0849
Nearest person month worked:	4 calendar months
Contribution to Project:	Performed initial in vitro experiments on macrophages for Specific Aim 3.
Funding Support:	Biomedical Engineering Department at SBU and National Aeronautics and Space Administration

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization name: Stony Brook University

Location: Stony Brook, New York

Partner's contribution to the project: We are working in collaboration with Dr. Judex at SBU to manufacture a device to deliver LIV locally to injured tissue and to accomplish the tasks in Specific Aim 3. The local LIV device is being manufactured at SBU and they have carried out the initial in vitro experiments for Specific Aim 3 in macrophages.